Research Article

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EVALUATION OF COMBINED EFFECT ON ANTIHYPERLIPIDAEMIC ACTIVITY OF *MADHUCA LONGIFOLIA* AND *BUTEA MONOSPERMA* L. IN TRITON WR-1339 INDUCED HYPERLIPIDAEMIC RATS

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ABSTRACT

The use of herbal remedies in India has a long history for various medical conditions. High serum total cholesterol, low-density lipoprotein (LDL) concentrations, and a decline in high-density lipoprotein are the main risk factors for coronary heart disease. It has been observed that *Butea monosperma* L. flower extract and *Madhuca longifolia* bark extract have been used traditionally to treat hyperglycaemia and related hyperlipidaemia. The evolution of atherosclerotic plaques and coronary artery disease are known to be accelerated by hyperlipidaemia. In the current study, hyperlipidaemic rats were used as a model to examine the potential antihyperlipidemic effects of a hydroalcoholic extract of *Butea monosperma* L. flowers (HA-BM) and *Madhuca longifolia* bark (HA-ML). Triton WR 1339 (400 mg/kg) was administered intraperitoneally (*i.p.*) once to rats to cause hyperlipidaemia, which resulted in rats with persistently high serum levels of triglycerides and cholesterol. The combined dose of HA-BM (200, 400 and 600 mg/kg/day) and HA-ML (250, 500 and 750 mg/kg/day) at a ratio of 1:1 was given to normal and hyperlipidaemic rats. Serum and liver tissue were analysed for lipid profile, and the activity was compared to the cholesterol-lowering drug, atorvastatin (10 mg/kg). From the above study, it could be concluded that a combined dose of an extract of *Butea monosperma* L. and *Madhuca longifolia* not only resulted in a significant reduction in cholesterol, triglyceride, LDL, VLDL level but also increased the HDL level, which is good for health.

Keywords: *Butea monosperma* L, *Madhuca longifolia*, Hyperlipidaemia, Coronary artery disease.

INTRODUCTION

Hyperlipidaemia is a diverse group of diseases that can be classified as primary or secondary based on their aetiology. Primary hyperlipidaemia can be caused by a single inherited gene defect or a combination of genetic and environmental factors. Secondary hyperlipidaemias are caused by a more widespread metabolic disorder, such as diabetes, excessive alcohol consumption, hypothyroidism, or primary biliary cirrhosis. [1-2] Hyperlipidaemia is a condition in which there is an excess of fatty substances called lipids in the blood, primarily cholesterol and triglycerides. Because these fatty substances travel in the blood attached to proteins, it is also known as hyperlipoproteinemia. This is the only way to dissolve these fatty substances in the bloodstream. [3] Excess lipids or fatty substances in the blood cause hyperlipidaemia, a significant risk factor for developing atherosclerosis and heart disease. In the blood, lipids take the forms of cholesterol, triglycerides, and lipoproteins, which are fat and cholesterol molecules linked to protein. Lipoproteins are classified into three types: very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and intermediate-density lipoproteins (IDL). Chylomicrons are lipoproteins made up of triglycerides, cholesterol, and protein. There are also high-density lipoproteins (HDL) that are inversely related to the risk of heart disease and are thus referred to as "anti-risk" factors.[4]

Herbal medicines have been traditionally used to treat various diseases without any side effects. One of the numerous herbal medicines with the potential to treat a wide range of ailments is the combination of *Madhuca longifolia* and *Butea monosperma*. The study aimed to examine the potential antihyperlipidemic and antioxidant effects of a hydroalcoholic extract of *Madhuca longifolia* and *Butea monosperma* L. in hyperlipidaemic rats.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Bark of *Madhuca longifolia* and flowers of *Butea monosperma* L. were collected from Yavatmal, Dist. Yavatmal, Maharashtra, India. The plant material was deposited and authenticated by Dr Panjabrao Deshmukh Krishi Vidyapeeth, Vasantrao Naik College of Agricultural Biotechnology, Waghapur Road, Yavatmal, with specimen number 12261/12252.

Preparation of Plant Extracts

Both plant materials were grounded into powder and dried in the shade. The 500 gm of Bark of *Madhuca longifolia* powdered material and flowers of *Butea monosperma* L were macerated for a week before hydroalcoholic extraction to obtain a residue.[5] The extract was evaporated to dryness. The creation of various fractions from the whole hydroalcoholic extract using the maceration process and increasing polarity of solvents (e.g., petroleum ether) is known as separation.[6]

Experimental Animals

Stable Sprague Dawley Rats (8 weeks old) weighing 150 and 250 gm were chosen for the investigation. The animals were kept in regular environmental conditions (temperature 22+ 2 °C, relative humidity 55-60%) in polypropylene cages with wire mesh and husk bedding.[7] Over the trial, rats received a regular pellet diet

from Amrut Feeds, Sangli, Maharashtra, and unlimited access to tap water. The rats were housed and cared for according to CPCSEA and IAEC guidelines. The protocol for the study was approved by the Institutional Animal Ethical Committee (IAEC) with reference no. 650/PO/Re/S/2002/CPCSEA/2021/09.

Experimental Design

For this study, animals were divided into six groups, each group containing six animals. [8-9]

Group I - Control group (Positive Control)
Animals were not treated with any drugs. Only 5% of CMC was given during the study.

Group II - Negative Control Group: Animals were treated with by single *i.p.* dose of Triton WR 1339 (400 mg/kg) only.

Group III - Triton WR 1339 (400 mg/kg, *i. p.*) + Hydroalcoholic extract of BM 200 mg/kg per day + ML 250 mg/kg per day was suspended in 5% CMC and was administered by oral route for 14 days to the Triton WR 1339 treated rats.

Group IV - Triton WR 1339 (400 mg/kg, *i. p.*) + Hydroalcoholic extract of BM 400 mg/kg per day + ML 500 mg/kg per day was suspended in 5% CMC and was administered by oral route for 14 days to the Triton WR 1339 treated rats.

Group V - Triton WR 1339 (400 mg/kg, *i.p.*) + Hydroalcoholic extract of BM 600 mg/kg per day + ML 750 mg/kg per day was suspended in 5% CMC and was administered by oral route for 14 days to the Triton WR 1339 treated rats.

Group VI- Triton WR 1339 (400 mg/kg, *i.p.*) + Atorvastatin (10 mg/kg/*p.o.*) was given to the Triton WR 1339 treated rats for 14 days.

To induce hyperlipidaemia in the rats, a single dosage of Triton WR-1339 (400 mg/kg body weight) *i.p.* was given to the rats. [10] After 72 hours, 5% CMC, BM extract, ML extract, and Atorvastatin were administered to the respective groups of rats.

Biochemical Studies

On days 1, 8, and 14, the blood samples were collected by retro-orbital plexus in rats under light ether anaesthesia. [11] Blood samples were taken while lightly sedated. To obtain serum, collected blood samples were centrifuged in a cooling centrifuge (2500 rpm for 10 min). The serum lipid parameters were measured using commercially available kits from after (Ambica diagnostic kits) [12]. The livers of each animal from all six groups were isolated and the liver extracts were evaluated for lipid profile and antioxidant potential. The Friedewald formula was used to determine the serum levels of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). [13-15]

Histopathological Studies

The animals were sacrificed for histopathology studies at the end of the study period. The livers of each animal from all six groups were isolated for histopathology study.[16] The isolated livers were carefully kept in a 10 % formalin solution to prevent damage.

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed and compared using one-way analysis of variance (ANOVA) to evaluate the lipid-lowering activity of the hydroalcoholic extract of *Madhuca longifolia* and *Butea monosperma* L. The study parameters were statistically analysed and compared by using the Dunnett test. [17]

RESULT

Combined Effect of HABM and HALM on Serum and Liver Lipid Profile

Compared to the normal control group, Triton WR 1339 treatment resulted in a substantial increase in the amount of serum CH, TG, LDL, and VLDL and a significant decrease in the level of serum HDL. Treatment with HAML-(250, 500 and 750 mg/kg/day) and HABM (200, 400 and 600 mg/kg/day) considerably decreased the level of serum and liver CH, TG, LDL, and VLDL and significantly elevated the level of serum and liver HDL when compared to the Triton WR 1339-treated group. (Figures 1 and 2).

Combined Effect of HABM and HALM on Antioxidant Potential in Hyperlipidemic Rats after 14 Days

The administration of Triton WR 1339 injections dramatically reduced the activity of the liver's SOD, CAT, reduced glutathione (GSH), and GPx antioxidant enzymes when compared to the healthy control group (Figure 3). However, treatment with HAML-(250, 500 and 750 mg/kg/day) and HABM (200, 400 and 600 mg/kg/day) effectively mitigated the decline in the activities of antioxidant enzymes SOD, CAT, GSH, and GPx in a dose-dependent manner when compared to Triton WR 1339-treated group.

Combined Effect of HAML-HABM on Histopathology of Liver

Based on the degree of the alterations, the histological examination of the liver was assessed and rated. (Figure 4)

Control group; normal architecture; no inflammation, fibrosis, fatty changes and necrosis.

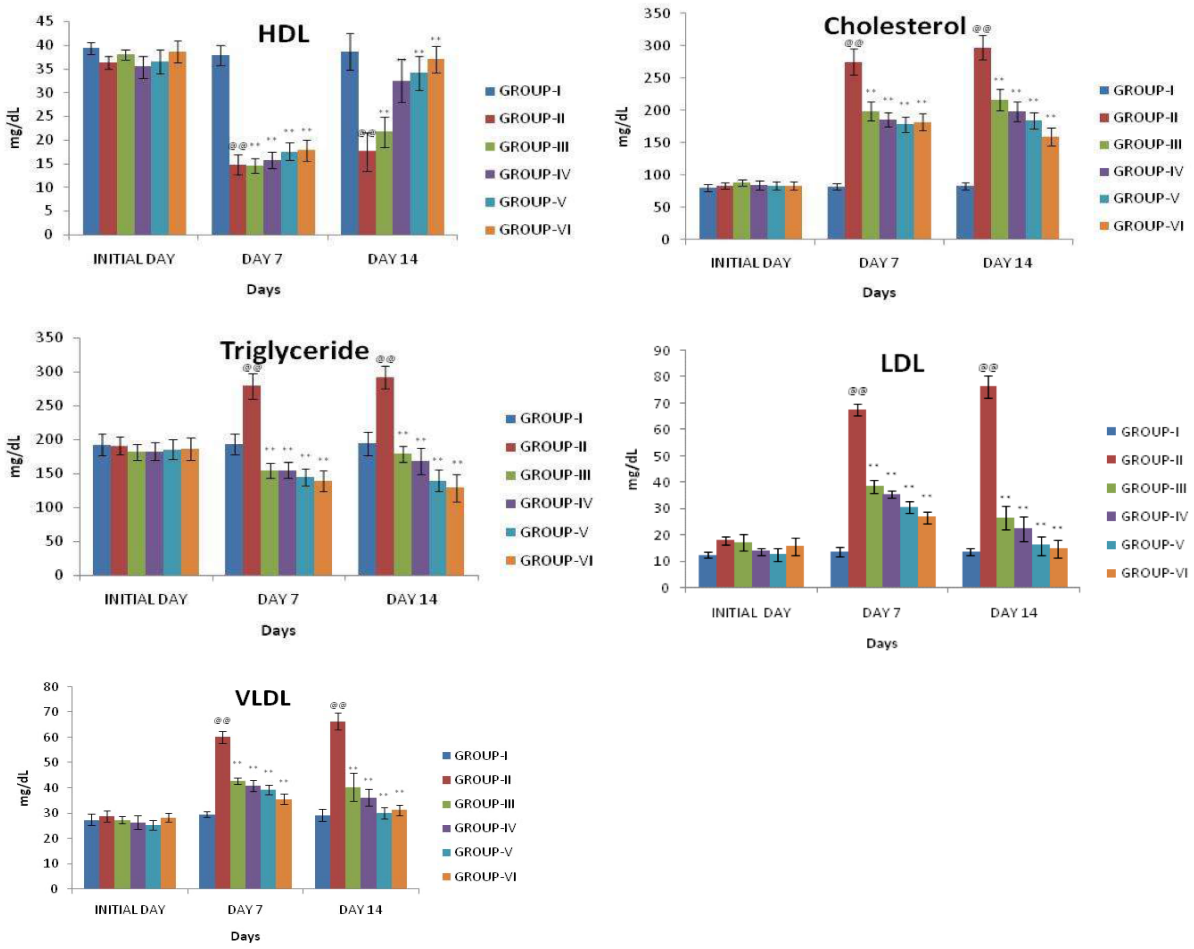
Hyperlipidaemic group showing; altered architecture. Hepatocytes focally show fatty vacuoles and focal lymphocytic infiltration around bile ductules. Fatty vacuoles push the nucleus to one side without fibrosis, inflammation or necrosis. This feature favour fatty changes in the liver with fatty infiltration and granular degeneration.

The group treated with HAML 250 mg/kg + HABM 200 mg/kg showed moderate cytoplasmic fatty infiltration and moderate granular degeneration.

The group treated with HAML 500 mg/kg + HABM 400 mg/kg showed moderate cytoplasmic fatty infiltration and mild granular degeneration.

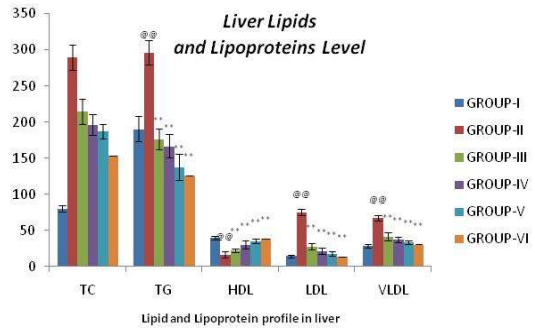
The group treated with HAML 750 mg/kg + HABM 600 mg/kg showed mild cytoplasmic fatty infiltration and mild granular degeneration.

No alterations were observed in the liver histology for the group treated with the standard drug atorvastatin, which showed negligible cytoplasmic fatty infiltration and granular degeneration.



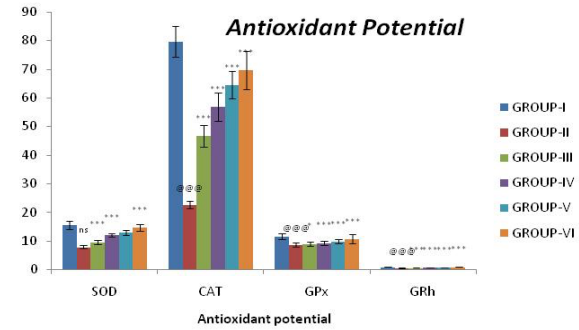
Values are expressed in Mean±SD, (n=6) p<0.001, p<0.01, p<0.05 compared with Group I; **p<0.001, **p<0.01, p<0.05 compared with Group II

Figure 1: Combined Effect of HAML-HABM on Serum Lipids and Lipoproteins Level in Hyperlipidemic Rats



Values are expressed in Mean±SD, (n=6) p<0.001, p<0.01, p<0.05 compared with Group I; **p<0.001, **p<0.01, p<0.05 compared with Group II

Figure 2: Effect of HAML-HABM on Liver Lipids and Lipoproteins Level in Hyperlipidemic Rat liver after 14 Days



Values are expressed in Mean±SD, (n=6) p<0.001, p<0.01, p<0.05 compared with Group I; **p<0.001, **p<0.01, p<0.05 compared with Group II.

Figure 3: Effect of HAML-HABM on Antioxidant Potential in Hyperlipidaemic Rats after 14 Days

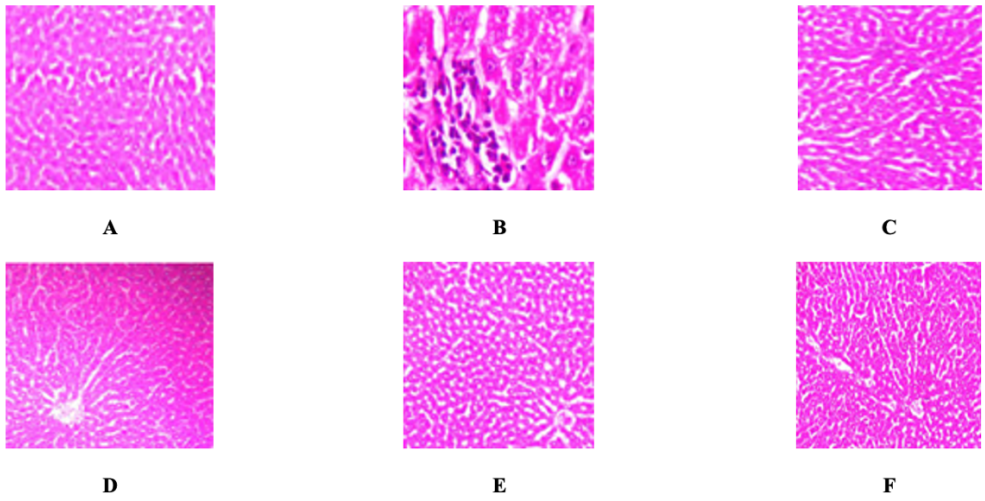


Figure 4: Combined Effect of HAML-HABM on rat liver (A: Normal rats, B: Hyperlipidaemic rats, C: HAML 250 mg/kg + HABM 200 mg/kg, D: HAML 500 mg/kg + HABM 400 mg/kg E: HAML 750 mg/kg + HABM 600 mg/kg, F: Atorvastatin 10 mg/kg)

DISCUSSION

The current study aims to evaluate the combined antihyperlipidemic efficacy of HAML and HABM in Triton WR 1339-treated hyperlipidaemic rats. There is an urgent need for the development of safe hypolipidemic medications from natural resources, and there has been a lot of interest in creating novel drugs from these plant materials to replace synthetic drugs due to their adverse effects. Hyperlipidaemia and oxidative stress have been considered more prominent causative factors for developing cardiovascular diseases such as atherosclerosis, acute myocardial infarction, hypertension, and coronary heart diseases.[18-19] There has been considerable interest in finding new drugs from plant materials to replace synthetic drugs due to their adverse effects, and there is an urgent need for the development of safe hypolipidemic drugs from natural resources. Triton WR-1339 (tyloxapol) is a non-ionic surfactant extensively employed to investigate the hypothetical mode of action of lipid-lowering drugs/molecules. After the injection of TritonWR1339, there were significantly elevated serum cholesterol levels, triglyceride, and lipoproteins.[20] HAML and HABM extracts were suspended in 5% CMC and administered to Group III to Group V as per the study schedule. The current investigation results indicate that the combined effect of HAML and HABM has significant potential to lower plasma lipid levels by reducing oxidative stress in hyperlipidaemic rats. Thus, ML and BM may be excellent herbal candidates for treating cardiovascular diseases and complications without adverse effects.

CONCLUSION

The current study provides strong evidence that combined intragastric administration of HAML (250, 500 and 750 mg/kg/day) and HABM (200, 400 and 600 mg/kg/day) has a beneficial effect in treating dyslipidaemia with a decrease in oxidative stress. Here we may conclude that HAML, in combination with HABM, may be helpful in the treatment and management of hyperlipidaemia along with oxidative stress.

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